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# High-throughput screening of excipients with a biological effect: a kinetic study on the effects of surfactants on efflux-mediated transport

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**Keywords**
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## Abstract

**Objective** In this study, we develop and apply a high-throughput screening protocol to investigate the activity of non-ionic surfactants, with a broad range of hydrophilic–lipophilic balance values, against ABCB1-mediated efflux transport and ABCC2-mediated efflux transport.

**Methods** Caco-2 cells were grown for 7 days in 96-well plates, then washed and incubated with the test materials for 2 h in the presence of 2.5 μM of either rhodamine 123 (R-123) or 5(6)-Carboxy-2′,7′ dichlorofluorescein diacetate as probes of ABCB1 and ABCC2, respectively.

**Key findings** Of the surfactants tested, no activity against ABCC2 was detected and all surfactants showing efficacy against ABCB1 had a HLB value of 22 or below. Inhibition of ABCB1 was seen in the order of efficacy to be poloxamer 335 > poloxamer 40 > Crovol A-70 > Myrj S-40 > poloxamer 184 > poloxamer 182 > Etocas 40 > Tween 20 > Etocas 29 > Tween 80 > Acconon C-44 > Span 20. With regard to this inhibition, the distribution of hydrophilic regions is more important than the HLB value.

**Conclusion** This work demonstrates a high-throughput protocol for detecting materials that can modulate ABCB1-mediated efflux. These surfactants could be exploited to improve oral delivery of drugs prone to efflux.

## Introduction

Pharmaceutical compounds administered orally must pass a series of barriers to reach systemic circulation. The intestinal wall, particularly the jejunum, represents the final obstacle, coinciding with a high concentration in expression of efflux transporters. These include the major adenosine triphosphate (ATP)-binding cassette (ABC) transporters most notably ABCB1 (P-gp) and the multidrug resistance protein (MRP2, ABCC2)[1] which expel a broad range of drugs back in to the intestinal lumen, thereby limiting their bioavailability.

While specific inhibitors of ABCB1 and ABCC2 exist, many act at such low efficacy that unrealistically high clinical concentrations are required to have an impact *in vivo*. Furthermore, many of these inhibitors have their own undesirable pharmacology. There are numerous reported examples of excipients (many of which are surfactants) that can inhibit efflux transporters. Indeed, the effects of surfactants on efflux-mediated transport first appeared in the literature with regard to the reversal of multiple drug resistance, the phenomenon by which solid tumour cells become refractory in nature by the upregulation the *MDR1* gene encoding P-glycoprotein'.[2] This was demonstrated when the polyethyloxlated castor oils Cremophor EL sensitised the MDR cell lines R100 and K562 to the chemotherapeutic drug daunorubicin.[3] Since then, a number of surfactants have been shown to be inhibitors of ABCB1-mediated transport, including Pluronic P85,[4] D-α-tocopheryl polyethylene glycol succinate (TPGS)[5] and Tween 20, Tween 80, Myrj 52 and Brij 30.[6]

In contrast, the impact of surfactants on ABCC2-mediated transport is less well studied. Cremophor EL, Cremophor RH 40, Tween 80 and Vitamin E TPGS 1000 have been shown to decrease ABCC2-mediated transport of anion calcein on MDCKII-ABCC2 cells.[7] In another study, SF9 membrane vesicles overexpressing ABCC2 were used to show that Cremophor EL, Cremophor RH 40,

Pluronic F127 (poloxamer 407), Pluronic F68 (poloxamer 188) and Capmul MCM were able to inhibit MRP2-mediated transport of scutellarin.[8] A subsequent study by the authors found that the inhibition of ABCC2 by the polyethoxylated castor oils was concentration-dependent.[9] However, the distinction between excipients which inhibit ABCB1 and those which inhibit ABCC2 remains unclear.

To investigate the potential of surfactants to interact with these transporters, we have used polarised and differentiated adenocarcinoma (Caco-2) cells. Caco-2 cells are highly regarded as the *in-vitro* 'gold standard' model for the small intestine, expressing microvilli and enzymatic components resembling those of the intestinal epithelium.[10] The cell line is well characterised and has long been established as a platform to study ABCB1-mediated transport. More recently, the Caco-2 cell line has also become a platform for the study of ABCC2-mediated transport.[11] There are a variety of in-vitro models that can be employed for screening ABCC2 activity using Caco-2 cells, with cells grown on permeable inserts, such as the widely used Transwell® model system, being commonly used as a model for intestinal drug absorption. Although a powerful screening tool, the Transwell® model suffers from slow growth rates that limit throughput. As such, we have adopted a novel assay using the Caco-2 platform grown in 96-well plates to achieve high-throughput screening (HTS) to assess the intracellular uptake of the fluorescent dye rhodamine 123 (R-123) as a high-affinity ABCB1 probe substrate dye and 5 (6)-carboxy-2′,7′-dichlorofluorescein as the high-affinity ABCC2 probe substrate. This HTS protocol offers a number of advantages over the Transwell® model including reduced growth time, fast processing time, low unit cost, in a multipurpose platform that can easily be used for fluorescent probe uptake as well as cytotoxicity and morphological studies without modification. In this study, we present a kinetic evaluation on the impact of surfactants on ABCB1-mediated transport and ABCC2-mediated transport using the Caco-2 cell line in this novel high-throughput system, with the aim of modelling such materials as drug–drug interactions.

## Materials and Methods

### Materials

Caco-2 cells (CACO-2; ECACC 09042001) (Public Health England, Porton Down, Salisbury, UK) were purchased from Public Health England and used at passage numbers 49-55. L-glutamine, fetal calf serum (FBS), Dulbecco's modified Eagle's medium (DMEM) and non-essential amino acids (NEAA) were purchased from BioSera (East Sussex, UK). Ciclosporin (CsA), verapamil (VER), indometacin (IND), probenecid (PRO), rhodamine 123 (R-123), 5(6)-Carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA), MTT, poloxamer 407, Tween® 80 and Tween® 20 and 0.5% trypsin-EDTA were purchased from Sigma-Aldrich (Dorset, UK). Capmul® MCM, Capmul® MCM C8-2, Capmul® PG-12, Capmul® PG-8, Acconon® C-44 and Acconon® MC-8 were a kind gift from ABITECH Corporation (Columbus, USA). Poloxamer 182, Span™ 20, Arlatone™ TV, Crovol™ A70, Etocas™ 29, Brij™ S-10, poloxamer 184, Etocas™ 40, Brij™ CS-12, NatraGem™ S140, Cetomacrogol™ 1000, Myrj™ S40, poloxamer 335, Myrj™ S100, poloxamer 407 and poloxamer 188 were gifts from Croda (Goole, UK).

### Stock solutions

Stock solutions of excipients were made in fresh Krebs–Henseleit (KH) buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.2 mM $KH_2PO_4$, 25 mM $NaHCO_3$, 11 mM glucose) to a final concentration of 10% (w/v). Fluorescent probes were prepared to 20 μM in DMSO and stored at −80°C until use. Experimental use of DMSO never exceeded 0.0125% (v/v), which was not considered to have a significant impact on the study.

### Cytotoxicity and inhibitor activity testing of excipients

Cytotoxicity and cellular accumulation of tracer dye studies were studied in Caco-2 cell. Cells were seeded in clear 96-well plates to a density of $4 \times 10^4$ cells per well and grown for 5–7 days. Cells were grown at 37°C in 5% $CO_2$ with 90% relative humidity in DMEM supplemented with 20% fetal bovine serum (FBS) for seeding media or 10% FBS for growth media, 1% non-essential amino acids (NEAA) and 2 mM L-glutamine and in the absence of antibiotics. Media was renewed every 48 h. Confluence was confirmed using light microscopy.

To determine an appropriate non-toxic concentration range to use for testing of the excipients, mitochondrial activity was measured by the reduction in MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) to the insoluble product formazan. Culture media of confluent 96-well plates was exchanged for 200 μl test solution containing increasing concentrations of the excipient or drug solutions and incubated for 4 h to encompass an incubation period longer than operational exposure time of the cells during assay conditions. The wells were then emptied and refilled using 100 μl of 0.5 mg/ml MTT and incubated for 3 h. The formazan was then dissolved using 100 μl of 0.1 N HCl in isopropanol and absorbance then read at 570 nm. Non-treated control wells were set to 100% viability.

To assess inhibitor activity of excipients against ABCB1 and ABCC2, the cellular accumulation of tracer dyes was employed; dye rhodamine 123 (R-123) was used as a high-affinity ABCB1 probe substrate and 5(6)-carboxy-2′,7′-dichlorofluorescein (CDFDA) as the high-affinity ABCC2 probe substrate. As with the MTT assay, Caco-2 cells seeded to a density of $4 \times 10^4$ cells per well and grown for 5–7 days were employed. To test inhibitor activity, the monolayers were washed twice using KH (pH 7.4) and left to equilibrate for up to 30 min. The wells were then refilled using 200 µl KH buffer containing the excipient of study over a logarithmic concentration range. Following 30 min pre-incubation with the selected excipient or inhibitor, the wells were emptied and refilled with the same concentrations of excipient or inhibitors mixed with 2.5 µM of either R-123 or CDFDA. This step was necessary in order to negate the influence of excipient viscosity acting to prevent dye ingress. Following 2 h incubation with the transporter probes (37°C in the dark), the cells were washed three times using ice-cold water and snap-frozen at −80°C for 5 min or overnight. Cells were then lysed in double distilled water under refrigeration and the lysate was transferred to black 96-well plates for fluorescence reading (ex 485 nm, em 535 nm).

### Calculation of kinetic parameters

To quantify the biological effects of excipients, the half maximal inhibitory concentration ($IC_{50}$) values were determined using GraphPad Prism (Version 6.0, GraphPad Software, Inc., San Diego, CA, USA) and represent the concentration of the tested compound capable of reducing transporter activity by 50%. Data were normalised against control cells that were incubated with the appropriate tracer dye but in the absence of an inhibitor, which were set to 100% efflux activity. These $IC_{50}$ values were then used for direct comparison to known inhibitors of efflux transporters.

### Statistical analysis

All data sets were assessed for significance using analysis of variance (ANOVA) with a predetermined alpha value of 0.05 using GraphPad Prism (version 6.0) with Dunnett's multiple comparison post-test for one-way and two-way ANOVA. In all figures, the error bars depict standard deviation.

## Results and Discussion

### Simple and rapid measurement of inhibitor activity of the ABCB1-mediated transport and ABCC2-mediated transport

To establish the functional activity of the transporters, recognised inhibitors of the efflux transporters were tested in conjunction with the fluorescent probes. Rhodamine 123 (R-123) and 5(6)-Carboxy-2′,7′-dichlorofluorescein diacetate (CDFDA) were used as a high-affinity ABCB1 and ABCC2 probe substrates, respectively. At low concentrations, the uptake of R-123 has been shown to be a saturable process that can be inhibited by the organic anion-transport (Oat)1a4 substrates digoxin, quinine, among others, in the rat model.[12] At higher concentrations, the uptake of R-123 is by passive diffusion. As an amphiphilic molecule, R-123 is able to traverse the lipid bilayer by inversions known as 'flip flop' events. R-123 excluded by P-gp is free to continuously re-enter the cell via a process by which the hydrophobic elements of the compound initially bind to the acidic head groups of the phospholipids. The efflux of R-123 by P-gp is mediated by pairs of the probe binding simultaneously to the protein.[13] Although MRP1 has been suggested to have a role in R-123 transport,[14] expression of this efflux transporter is low on the Caco-2 cell line,[15] and hence specificity of the probe to P-gp. Only mutated BCRP is capable or transporting R-123 while the wild-type BCRP cannot.[16]

The results shown in Figure 1 demonstrate that the inhibitors of both ABCB1 (VER and CsA) and ABCC2 (IND and PBD) follow a dose-dependent response (non-linear regression curves), allowing $IC_{50}$ values to 95% confidence to be calculated. The ABCB1 inhibitors VER and CsA give $IC_{50}$ values of 4.08 and 1.16 µM, respectively, with the ABCC2 inhibitors IND and PBD giving $IC_{50}$ values of 38.08 and 68.08 µM, respectively (Figure 1). Compared to established literature (Table 1), the derived $IC_{50}$ data for the ABCB1 inhibitors align well with the published range. For the inhibitors of ABCC2, the derived $IC_{50}$ value for IND also aligns well with published literature, although the derived value for PBD is an order of magnitude lower than the published values (Table 1). However, comparative literature $IC_{50}$ values for IND and PBD as inhibitors of ABCC2-mediated efflux are limited, despite their prevalence in the literature as common modulators of this transporter. In this respect, the study by Heridi-Szabo *et al.* was used to derive two further $IC_{50}$ values from the $K_i$ values given.[17]. These $K_i$ values were converted to $IC_{50}$ values using the Cheng–Prusoff equation[18] shown in Equation 1,

$$K_i = \frac{IC_{50}}{1 + \frac{[S]}{Km}} \quad (1)$$

where $K_i$ is the inhibitor affinity, the $IC_{50}$ is the concentration of inhibitor that inhibits 50% of the transport, $[S]$ is the concentration of the substrate and $K_m$ is the substrate affinity.

From the published values collected in Table 1,[17,19–28] it is apparent that the type of probe and inhibitor combination used has a notable impact: Rautio *et al.* used the Caco-

2 platform in the Transwell® format and evaluated the $IC_{50}$ values of VER using multiple probe substrates.[17] The inhibition $IC_{50}$ value of VER was found to be below 11 μM when digoxin and prazosin were used as probe substrates, between 17 and 34 μM when colchicine and vinblastine were used as probe substrates, and 60.9 μM when calcein-AM was used as a probe substrate (Rautio *et al.*, 2006). As P-gp has known multiple binding sites,[27,28] Rautio *et al.* attributed the broad range of inhibition $IC_{50}$ values to the different inhibitors exerting their effects on different binding sites within the transporter.[19] Taken together, the data present in Figure 1 and Table 1 confirm the functionality of the ABCB1 and ABCC2 transporter systems on the cell line by using the combination of specific fluorescent probes with established inhibitors, subsequently confirming that the high-throughput 96-well method can be used to measure inhibitor activity of the ABCB1-mediated transport and ABCC2-mediated transport.

## The effects of surfactants on ABCB1-mediated efflux and ABCC2-mediated efflux

To examine the underlying structural relationship between surfactants and efflux inhibition, a broad range of compounds encompassing different HLB values, molecular weights and structural composition were screened (Table 2). In general, surfactants have a high propensity to interact with a lipid bilayer, and in doing can eventually breach the cell membrane and cause loss of cellular viability. As such, all surfactants were tested using the MTT assay to confirm an appropriate concentration range for further testing (Figure S1).

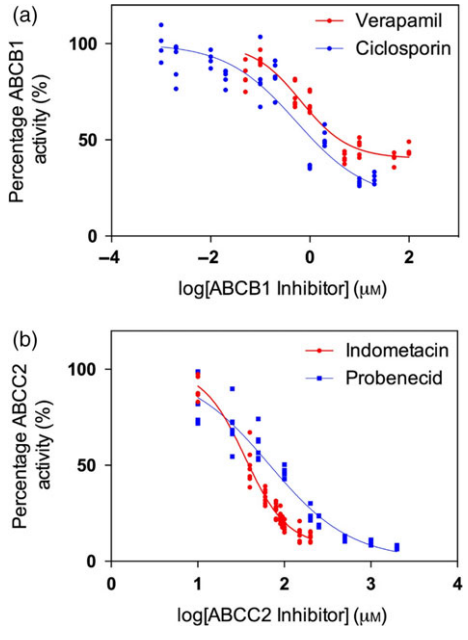


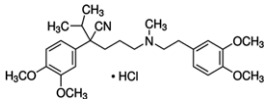
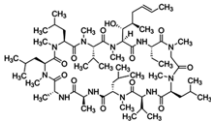
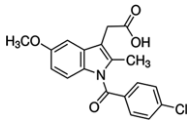
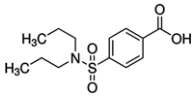

**Figure 1** The $IC_{50}$ curves of inhibitors of efflux proteins, showing activity against (a) ABCB1 and (b) ABCC2. Caco-2 cells were grown for 5–7 days on 96-well plates before experimentation. Data presented as each of a minimum of four replicates from 2.5 μM initial extracellular concentration of either R-123 or CDFDA as probes of ABCB1 and ABCC2 activity, respectively.

Of the 24 surfactants tested, 12 yielded data from which $IC_{50}$ values could be determined for ABCB1 inhibition with over 95% confidence (Figure 2) with the remaining excipients correlating with linear regression and therefore showing no efflux activity (Table 2). Across the surfactants tested, only activity against the ABCB1 was detected and these are shown in Figure 2. Against ABCB1, the excipients are rank ordered: poloxamer 335, poloxamer 407, Crovol A-70, Myrj S-40, poloxamer 184, poloxamer 182, Etocas 40, Tween 20, Etocas 29, Tween 80, Acconon C-44 and Span 20. Of all the excipients, Span gave by far the highest $IC_{50}$ (7.7 mM; Figure 2a, Table 2). Excluding Span 20, the remaining excipients had $IC_{50}$ values of 26.5 μM or below (Table 2). The excipients with the lowest values, poloxamers 335 and 407, showed $IC_{50}$ values of 0.63 and 0.71 μM (Figure 2k and Figure 2l), exceeding that of VER (4.08 μM) and CsA (1.16 μM) (Figure 1a).

Despite the inherent biological effects of excipients being well established, supporting kinetic data in the literature are difficult to find. Tween 80 has been shown to have an $IC_{50}$ of 152 μM for inhibition against P-gp using *MDR1* overexpressing mouse lymphocytes (P388/mdr) using R-123 as a substrate.[29] The result of the present study (12.67 μM; Table 2) is much lower than the previously reported value and may be attributed to the low membrane partitioning of P388/mdr cells by Tween 80 which could be cell type specific. As in our study, Bogman *et al.* (2003) raised no significant modulation in the presence of poloxamer 188 to either ABCB1 or ABCC2.

The mechanism of action of these materials against ABCB1 is most likely a multifaceted process involving, among others, ATP depletion by the inhibition of ATPase[30,31] and/or membrane fluidisation.[6,32] Membrane fluidisation is a fundamental factor in efflux inhibition due to the sensitivity of ABC transporters to their microenvironment,[33] in particular P-gp ATPase activity (Regev *et al.*, 1999). Using porcine kidney epithelial cells overexpressing P-gp, MRP1 and MRP2, a similar study has examined Pluronic P85, similar in HLB to poloxamers 184 and 335 used in our study, as an efflux inhibitor.[31] The degree of ATPase inhibition was observed in the order MRP1 < MRP2 ≪ P-gp, attributed to conformation changes of the transporter via membrane fluidisation and/or steric hindrance of drug-binding sites by P85 side chains. Batrakova *et al.*[31] suggest that MRP structures may be more robust and resilient to alterations in fluidisation and

**Table 1** Literature $IC_{50}$ values of the inhibitors against those obtained from Caco-2 cells grown for 5–7 days on 96-well plates

| Drug | Structure | Transporter action | Reported $IC_{50}$ (μM) | | Mean determined value (μM) | Determined upper value (μM) | Determined lower value (μM) |
|---|---|---|---|---|---|---|---|
| Verapamil |  | ABCB1/P-gp | 1.18–60.9<br>4.2<br>6.5 | Rautio *et al.* (2006)[a]<br>Wang *et al.* (2001)[b]<br>Perloff *et al.* (2002)[c] | 4.08 | 7.44 | 2.36 |
| Ciclosporin |  | ABCB1/P-gp | 0.5<br>1.4<br>0.74–2.22 | Tamai and Safa (1991)[d]<br>Wang *et al.* (2001)[b]<br>Rautio *et al.* (2006)[a] | 1.16 | 1.936 | 0.72 |
| Indometacin |  | ABCC2/MRP2 | 25<br>46<br>75.1<br>99 | Colombo *et al.* (2012)[e]<br>El-Sheikh *et al.* (2007)[e]<br>Dahan and Amidon (2010)[c]<br>Heredi-Szabo *et al.* (2008)[e] | 38.99 | 41.4 | 36.31 |
| Probenecid |  | ABCC2/MRP2 | 130<br>812<br>814.8 | Colombo *et al.* (2012)[e]<br>Heredi-Szabo *et al.* (2008)[e]<br>Förster *et al.* (2008)[f] | 68.08 | 77.27 | 60 |

Data of determined $IC_{50}$ values show the outcome of the kinetic modelling using GraphPad prism presented in Figure 1 extrapolated for 50% transporter inhibition using the sigmoidal curve. [a]MDCKII-MDR1; [b]MDR1-NIH3T3-G185; [c]Caco-2; [d]Vincristine-resistant Chinese hamster lung cells; [e]MRP2 membrane vesicles; [f]MDCK-ABCC2.

also that the differences in the overall structures between P-gp and the MRP isoforms may also play a significant role by having a binding site which is inaccessible to the long side chains of the poloxamers.[31] Evers *et al.* could not find any correlation between P-gp and MRP inhibition using Pluronic L61 on MDCKII transfected cell lines.[34] This may explain why the excipients tested in this study yielded insufficient inhibition data to give an $IC_{50}$ value.

When considering the impact of the surfactant structural attributes, it has previously been noted that the inhibitory effects of poloxamers against ABCB1 are heavily dependent upon the HLB of the molecules.[31] In this comprehensive study, the block copolymers with intermediate propylene oxide blocks (30–60 units) with HLB values below 20 gave the optimum inhibition against P-gp in bovine brain microvessel endothelial cells (BBMECs) by increasing microviscosity of the lipid membrane and P-gp ATPase inhibition.[31] Indeed, our findings are in agreement. Of the five poloxamers tested, only P188 showed no pharmacological activity against ABCB1 and was more hydrophilic than the other poloxamers with a HLB of 29. Yet, closer examination of Table 2 reveals substantial gaps in the list that cannot be explained using HLB alone.

Of the materials tested, the lowest molecular weight surfactant used is Span 20, which has a comparatively high $IC_{50}$ value. Tween 20, with a molecular weight of 1128 Da, has an $IC_{50}$ value of 9.7 μM, yet has a very similar molecular weight to Cetomacrogol 1000 (1122 Da) which had no effect on ABCB1-mediated efflux (Table 2). For these materials, the separating factor appears to be the structural geometry. Tweens have central ring structure from which PEGylated moieties emanate, divided over three branches. For Cetomacrogol, the molecule is essentially split in two distinct regions, the hydrophobic part and the hydrophilic part in a linear fashion. As membrane interaction and subsequent fluidisation are the generally accepted mechanism of action, it can be expected that polarised linear chains represent a larger degree of steric hindrance during lipid partitioning compared to the compact geometry of Tween 20, through which membrane interactions can pivot around the hydrophobic ring system. Closely related to Cetomacrogol 1000, Brij CS-12 and Brij S-10 suffer the same fate. Myrj S-40 is also a linear chain, but appears to be able to interact with the membrane, and therefore inhibit, because of its more compact geometry with PEG 40 forming the hydrophilic element. In contrast, Myrj S-100 has no biological effect, most likely attributed to the extension of the linear chain by PEG 100 as the hydrophilic segment. Interestingly, Arlatone TV has no inhibitory effect, yet possesses six branches structure emanating from a sorbitol backbone. In this case, the distribution of the PEGylation appears inadequate (an average of 6.6 repeat units per branch) to counter the bulk of the molecule. Of all of the excipients screened, surfactants with intermediate HLB values that were small and linear or larger and branched showed high efficacy. It is of interest to note that this study

**Table 2** Physical properties and IC50 values against ABCB1 of the tested excipients

| Excipient | | Physical properties | | | $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|---|
| | | HLB[a] | MW | State | Average | Upper | Lower |
| Capmul PG-8 | Propylene glycol monocaprylate | 4.5 | 246[b] | Liquid | – | – | – |
| Capmul MCM | Medium chain monoglycerides and diglycerides | 5.5 | 218[b] | Liquid | – | – | – |
| Capmul MCM C8 | Glyceryl monocaprylate | 5.5 | 202[b] | Liquid | – | – | – |
| Capmul PG-12 | Propylene glycol monolaurate | 5.5 | 258[b] | Liquid | – | – | – |
| Poloxamer 182 | Poly(ethylene) Poly(propylene) glycol | 7.0 | 2500[a] | Liquid | 8.57 | 10.62 | 7.35 |
| Span 20 | Sorbitan monolaurate | 8.6 | 346[a] | Liquid | 7725.45 | 9793.18 | 6250.69 |
| Arlatone TV | PEG 40 sorbitan peroleate | 9.0 | 3508[b] | Liquid | – | – | – |
| Crovol A-70 | PEG 60 almond glyceride | 10.0 | 3251[b] | Liquid | 2.56 | 3.51 | 1.92 |
| Etocas 29 | PEG 29 castor oil | 11.7 | 2202[b] | Liquid | 12.00 | 17.87 | 8.84 |
| Brij S-10 | PEG 10 stearyl ether | 12.4 | 710[b] | Waxy | – | – | – |
| Poloxamer 184 | Poly(ethylene) Poly(propylene) Glycol | 13.0 | 2900[a] | Liquid | 7.64 | 10.11 | 5.95 |
| Etocas 40 | PEG 40 castor oil | 13.0 | 2686[b] | Liquid | 9.55 | 14.45 | 6.62 |
| Brij CS-12 | PEG 12 cetostearyl ether | 13.4 | 770[b] | Waxy | – | – | – |
| Acconon C-44 | Lauroyl macrogolglycerides | 13.5 | 1500[a] | Waxy | 26.54 | 32.88 | 21.62 |
| NatraGem S140 | Polyglyceryl-4 laurate/Sebacate | 14.0 | 664/532[b] | Liquid | – | – | – |
| Acconon MC8-2 | PEG 8 caprylic/Capric glycerides | 14.5 | 350[a] | Waxy | – | – | – |
| Tween 80 | PEG 80 sorbitan monolaurate | 15.0 | 1310[a] | Liquid | 12.24 | 19.89 | 7.44 |
| Cetomacrogol 1000 | PEG 20 cetostearyl ether | 15.7 | 1122[b] | Waxy | – | – | – |
| Tween 20 | PEG 20 sorbitan monolaurate | 16.7 | 1128[a] | Liquid | 9.65 | 12.76 | 7.41 |
| Myrj S-40 | PEG 40 stearate | 16.7 | 2044[b] | Solid | 6.13 | 8.84 | 4.62 |
| Poloxamer 335 | Poly(ethylene) Poly(propylene) glycol | 18.0 | 6500[a] | Solid | 0.63 | 0.75 | 0.53 |
| Myrj S-100 | PEG 100 stearate | 18.8 | 4684[b] | Solid | – | – | – |
| Poloxamer 407 | Poly(ethylene) Poly(propylene) glycol | 22.0 | 12 500[a] | Solid | 0.71 | 0.77 | 0.70 |
| Poloxamer 188 | Poly(ethylene) Poly(propylene) glycol | 29.0 | 8400[a] | Solid | – | – | – |

Molecular weight values were either obtained from the manufacturer or estimated from the structures, as given in the parentheses below. All other values were determined experimentally. [a]Determined by manufacturer. [b]Estimated from molecular weight.

puts a number of IC50 values for the excipients within the micromolar range and Crovol A70, poloxamer 335 and poloxamer 407 have efficacy that exceeds verapamil, while P335 and P407 exceed that of ciclosporin.

This study demonstrates that surfactants such as poloxamers and Tweens are able to modulate efflux-mediated transport. Due to the ubiquitous usage of many of these materials in oral dosage formulations, coupled with the substantial barrier shown by efflux transporters to many drugs, it is expected that the work described herein has widespread pharmacokinetic implications *in vivo*. Among others, poloxamers 407, Tween 20, Tween 80 and PEG 40 hydrogenated castor oil (analogous to Etocas 40 used in the present study) are widely used in oral formulations as solubilising agents, coatings and constituents of tablets. Both Gengraf® and KALETRA® oral solutions contain PEG 40 hydrogenated castor oil, one of the excipient inhibitors identified in the present study. Gengraf® is a formulation of ciclosporin, a known inhibitor of P-gp (Tamai and Safa, 1991) and also a substrate.[35] KALETRA® is an oral solution formulation of both lopinavir and ritonavir. Lopinavir has been shown to be an ABCB1 substrate *in vitro*[36–38] and also substrate of ABCC2.[36] Although ritonavir is an inhibitor of both ABCB1 and ABCC2,[39,40] this formulation also contains PEG 40 hydrogenated castor oil. Furthermore, KALETRA® 200 mg tablets contain sorbitan monolaurate (Span 20) in addition to polysorbate 80 (Tween 80) in the coating. One of the most potent inhibitors found in the present study, poloxamer 407, is found in many formulations including ISENTRESS® 400 mg film-coated tablets, containing raltegravir. This drug is an ABCB1 substrate in both CD4+ T cells[41,42] as well as human intestinal cells (Caco-2).[41]

To fully realise these properties, the excipients and the active compound must together reach the absorbing membrane, the intestinal epithelium. To achieve this, they must pass through the stomach. In a study of 12 volunteers, it was found that the stomach volume in the fasted state is as low as 13–72 ml.[37] With a medium-sized 500 mg tablet taken with 250 ml of water, the materials with the lowest $IC_{50}$ values in our data set, the poloxamers 335 and 407, would only need to be present at 1.3 and 2.8 mg, respectively, if taken on an empty stomach with 72 ml of fluid present. Even in the fed state, where a stomach can reach volumes of 534–859 ml,[37] these excipients would need to be present, theoretically, at 3.5 and 7.6 mg for poloxamers 335 and 407, respectively, for the higher stomach volume, or 1.5% (w/w) and 0.7% (w/w) of a medium-sized 500 mg tablet, respectively. As such, the data obtained in this study offer a selection of materials that can be

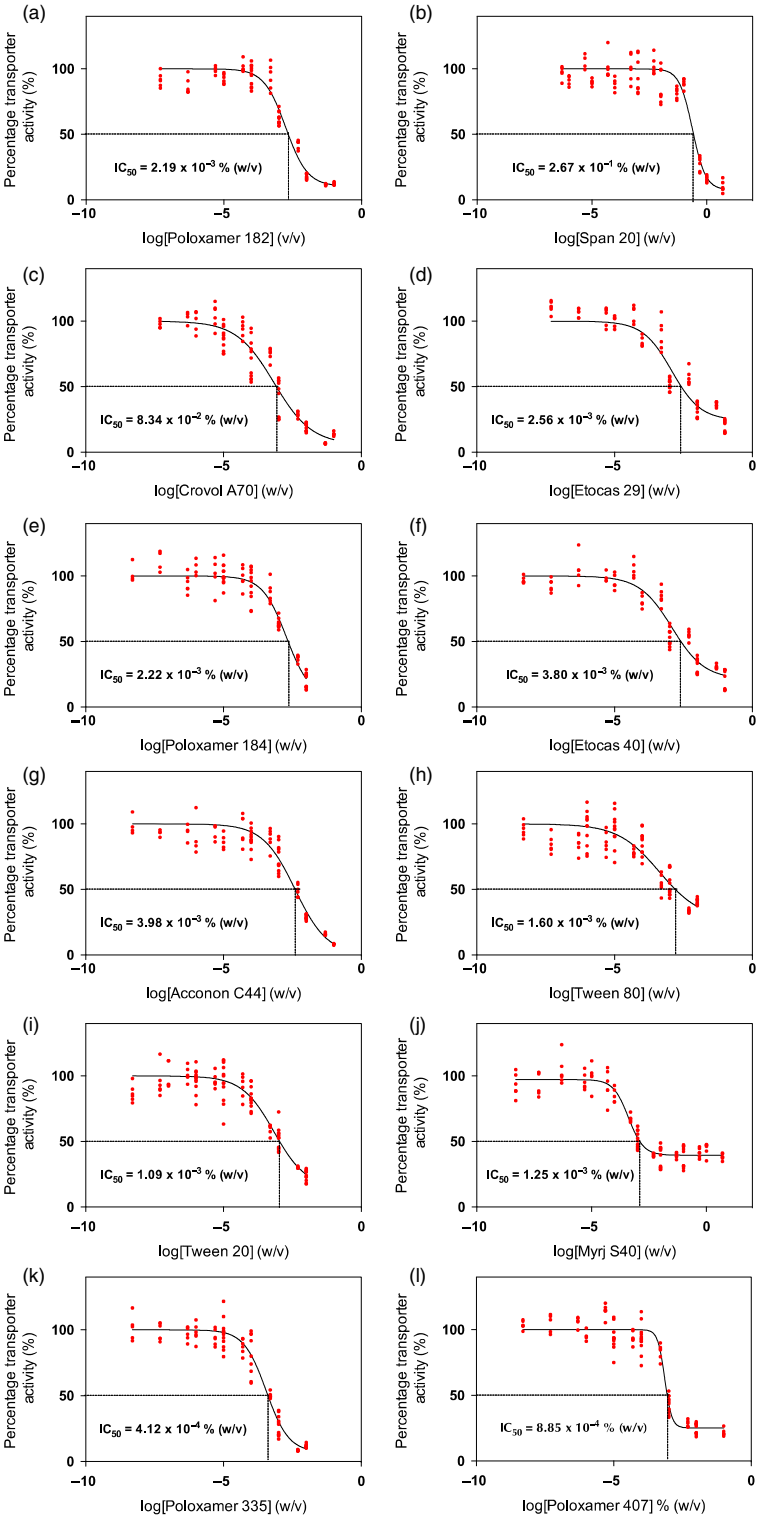



**Figure 2** Percentage inhibition curves of various surfactants against ABCB1 showing (a) Poloxamer 182, (b) Span 20, (c) Crovol A70, (d) Etocas 29, (e) Poloxamer 184, (f) Etocas 40, (g) Acconon C44, (h) Tween 80, (i) Tween 20, (j) Myrj S40, (k) Poloxamer 407 and (l) Poloxamer 335. Sigmoidal curves were constructed from four different passage numbers on confluent Caco-2 monolayers grown on 96-well plates for 5–7 days and are shown pooled from a minimum of four replicates per concentration. Data presented as a function of overall R-123 uptake for ABCB1 with initial conditions of 2.5 μM extracellular tracer dye per well, with excipient-free control wells set to 100% transporter activity.

incorporated into oral dosage formulations (potentially in combination to provide synergistic effects) for potential efflux inhibition *in vivo*. Furthermore, this work provides a high-throughput method for the identification of a working toolbox for the design and production of tablets with the potential to enhance delivery of drugs which are prone to efflux. This 96-well plate format may also be applicable to a wider range of cell models given its rapid and low-cost nature.

## Conclusion

Within this study, a broad range of excipients were screened using a new HTS assay for activity against ABCB1-mediated transport and ABCC2-mediated transport, with a number of inhibitors identified. The results presented in this study demonstrate the potential impact that surfactants could have on existing oral formulations. Moreover, these studies describe a series of excipients that are able to modulate efflux-mediated transport that could provide a unique method of increasing the uptake of drugs that are efflux substrates or provide a method for generic and innovator companies to achieve bioequivalence.

## Declarations

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1**. The effects of the surfactants on cellular viability using the MTT toxicology assay, showing A) Capmul PG-8, B) Capmul MCM, C) Capmul MCM C8, D) Capmul PG-12, E) Poloxamer 182, F) Span 20, G) Arlatone TV, H) Crovol A-70, I) Etocas 29, J) Brij S-10, K) Poloxamer 184, L) Etocas 40, M) Brij CS-12, N) Acconon C-44, O) NatraGem S140, P) Acconon MC8-2, Q) Tween 80, R) Cetomacrogol 1000, S) Tween 20, T) Myrj S-40, W) Poloxamer 335, X) Myrj S-100, Y) Poloxamer 407, Z) Poloxamer 188